STUDIES ON THE NERVE ENDINGS OF THE EXTERNAL GENITALIA OF THE BOVINE

by

V. P. RAO

B. V. Sc. University of Madras, 1943.

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Pathology

KANSAS STATE UNIVERSITY
OF AGRICULTURE AND APPLIED SCIENCE

1959

TABLE OF CONTENTS

INTRODUCTION

The gross anatomical structure of the external genitalia of the bovine has been described, at length, by Sisson (1953) and McLeod (1958). Most text books on histology deal with the microscopic structure of the genital system in the human. Trautmann and Fiebiger (1957) mention a few comparative features observed in ruminants. Trotter (1957) studied the gross anatomy and general histology of the male genitalia of the bovine. Mariappa (1957) described the histological structure of the glans clitoris of the Indian elephant. There is considerable information about the nerve endings, their occurrence and distribution in the human reproductive system. The literature contained only a generalization of the presence of special nerve endings in the genital organs of animals including the ruminant as outlined by Trautmann and Fiebiger (1957).

The main object of this work was to study the location of the various types of nerve endings and their distribution in the external genitalia of the bovine, both male and female. In the male the external genitalia included the penis, prepuce and the scrotum. In the female they included the clitoris, vestibule, labia, and the mammary glands. For the purpose of this work the study was confined to the glans penis, the anterior part of the body of the penis and prepuce in the male. In the female it included the clitoris, adjacent parts of the vestibule and the labia. Eight hundred and ninety two serial sections made at different levels of these organs of five males and seven females which ranged from 10 months to eight years in age were studied.

REVIEW OF LITERATURE

Nerve Supply. While understanding the part played by the arterial inflow in the mechanism of erection, the importance of nerve stimuli should not be overlooked. Ever since the classical demonstration of Eckhard that stimulation of certain nerves, nervi erigentes, in the dog caused an increase of blood flow through and enlargement of the penis, the arteries have been assumed to play an active role (Deysach, 1938). Fibers from the third and the fourth sacral nerves, nervi erigentes, passed with the sympathetic fibers to the erectile tissue and were said to be related to the act of erection (Lambert, 1944). The dorsal nerve of the penis, the continuation of the pudic nerve, supplied the ischiocavernosus muscle and the special nerve endings of the penis. The pudic nerve in the female terminated in the clitoris and vulva. The branches of the posterior mesenteric ganglion formed anastomoses with one another and with the branches of the third and fourth sacral nerves in the pelvic cavity, thereby resulting in peripheral plexuses. The cavernous plexus supplied the penis, or the clitoris and the utero-vaginal plexus, the uterus and the vagina (Sisson, 1953). The spinal, sympathetic and parasympathetic nerve fibers supplied the penis. The motor fibers of the sympathetic and the parasympathetic formed an extensive network in the walls of the blood vessels and the smooth muscles of cavernous trabeculae. The sensory fibers of the spinal nerves terminated in the free or non-encapsulated afferent endings in the epithelium of the glans, Meissner's corpuscles in the papillae of the prepuce and the glans; the end bulbs of Krause, the genital corpuscles of Dogiel and the Pacinian corpuscles (Copenhaver and Johnson, 1958).

Bohm et al. (1904) stated that the essential portion of a sensory nerve ending was a non-encapsulated telo-dendrion. These nerve endings occurred in all epithelial tissues and in fibrous connective tissues of certain regions. A large sensory nerve fiber, after coursing through the connective tissue, divided into a number of branches, always at the node of Ranvier. These workers continued by stating that these small medullated branches approached the epithelium, lost their sheaths, coursed between the epithelial cells, divided into smaller and smaller branches and finally terminated in small nodules or discs. Many other authors mentioned the occurrence of nerve endings in the epithelium and connective tissue. The simple tactile cells were oval, nucleated elements of five to 12 u, embraced by a crescentic expansion, the tactile membrane, with which the nerve fiber was probably connected. A compound tactile cell, the Corpuscle of Grandry, resulting from the association of two or more cells was found especially in birds and also in mammals as end plates of Merkel (Piersol, 1905). Sabotta (1930) found the free nerve endings chiefly in the stratified squamous epithelium but also in connective tissue membranes. In the cornea and epidermis, the nerve fibers lost their medullary sheaths before entering the epithelium. These naked axis cylinders penetrated even as far as the superficial layers. They terminated between the epithelial cells in slender points or button-like thickenings. In the epithelium covering the nose of many animals the intra-epithelial nerve endings had either small expansions with lateral branches terminating upon the epithelial cells or thin varicose fibers which passed between epithelial cells to form pericellular fibers resembling the tactile cells of Merkel.

Boeke (1932) said that the ultimate ramifications of the nerve fibers which became thinner and thinner, entered the epithelium as free axis cylinders and ended in either small knobs or a series of flattened leaf-like enlargements termed, "hederiform nerve endings" by Ranvier. When the flattened terminations were applied to the surface of somewhat enlarged and differentiated epithelial cells they formed the tactile cells of Merkel. Cutaneous pain stimulated these endings (Sanders, 1947). Ham (1953) stated that the external genitalia were rich in nerve endings.

Maximow and Bloom (1957) observed that the free nerve endings were present in the glans penis, prepuce and the urethra of the male and that they were scattered in the epithelium of the female genitalia. These authors stated that in the labia of the human female the nerve endings were circularly arranged in the middle layer of the dermal sheath and coursed parallel to the hair shaft in the outer root sheath of the hair follicle. Trautmann and Fiebiger (1957) expressed the belief that the free endings were receptors for pain, touch and various visceral sensations. Copenhaver and Johnson (1958) recorded that the free afferent endings were found in subepithelial tissue and in practically all epithelia but did not penetrate beyond the stratum granulosum in the skin. The hair follicles were encircled by the sensory fibers which terminated in the connective tissue sheath of the follicle to form the peritrichial ending.

Satterthwaite (1882) also found the Meissner's or the Wagner's corpuscles in the genitalia but said that they were only 0.1 mm long. Bohm et al. (1904) observed these corpuscles in the sub-epidermal connective tissue of the hand and foot and in the genital organs. They were

oval or irregular, measuring 100 to 180 u by 45 to 50 u. They had a thin connective tissue capsule with round or oval nuclei, which were sometimes oblique to the corpuscular axis. One, two, three or more nerves in the larger endings, lost their sheaths after piercing the capsule, branched and formed a complex network. One or several larger naked axis cylinders passed up through the axis of the spiral of fibers thus formed, gave off branches and contributed to the spiral formation. According to Piersol (1905) these corpuscles were oval, elliptical bodies, 45 to 140 u by 35 to 55 u in dimension and occurred in the papillae of the corium and in the external genitalia. The characteristic transverse or spiral markings were produced by the numerous transversely placed nuclei. One or two and sometimes three or four medullated nerve fibers made windings before entering the corpuscle. The nerve sheath became continuous with the envelope of the corpuscle. The nerve fibers retained their medullary sheath for a short distance but lost it later and divided into smaller non-medullated fibers which ended after a spiral course, in terminal discs. Sabotta (1930) described Meissner's or Wagner's corpuscles as elongated ellipsoidal bodies, 60 to 150 u by 30 to 60 u, with a distinctly striated appearance. They consisted of flat tactile cells piled up, one on the other, with flattened nuclei, between which were found the terminal fibrillar expansions of the axis cylinders of the nerve fibers. Two to five fibers entered the lower part of the corpuscle. The connective tissue sheath of the corpuscle was believed to have produced the superficial constrictions of the endings.

Boeke (1932) observed that the nerve fibers either at once or after winding around it two to three times, passed into the corpuscle and lost

their medullary sheath. The naked axis cylinders took a tortuous course through the core which was composed of flattened protoplasmic cells and ended in terminal enlargements. Ham (1953) observed that external genitalia were rich in nerve endings, and therefore rich in Meissner's corpuscles. Cauna (1956) pointed out that the nerve supply of a single Meissner's corpuscle might originate from one or two nerve bundles. The nerve fibers of one segment did not tresspass into the next across the transverse septum. Thus the receptor end of the tactile pathway in the finger pads was divided into units both by anatomical structure of the digital skin and by the pattern of nerve supply of Meissner's corpuscles.

Maximow and Bloom (1957) stated that Meissner's corpuscles occurred in the connective tissue of the fingers and toes, in the skin of prepuce and glans penis and also in the external genitalia of the female. They described them as being elongated, pear-shaped or elliptical in cutaneous papillae with the long axis perpendicular to the surface and measuring 40 to 100 u by 30 to 60 u. Meissner's corpuscles occurred as reported by Copenhaver and Johnson (1958) in the papillae of the external genitalia, especially on the hairless portions of the skin, such as finger tips and soles of feet and in the connective tissue of dermal papillae. These workers further stated that the corpuscles were oval bodies of connective tissue cells, arranged in horizontal lamellae. Two or more nerves entered each corpuscle after losing their sheaths, branched and took a spiral course within, eventually terminating in flattened expansions. They believed that these endings served tactile stimuli and were probably most concerned with discriminative touch.

The End Bulbs of Krause. These receptors were found to be ovoid in

shape and 0.05 mm in diameter and occurred in the external genitalia (Satterthwaite, 1882). Bohm et al. (1904) observed that these bulbs were round, oval or pear-shaped, the small ones were 0.02 to 0.03 mm by 0.015 to 0.025 mm and the larger ones 0.045 to 0.10 mm by 0.02 to 0.08 mm. The capsule was thin with numerous nuclei. One, two or even three medullated nerves lost their sheath on entering the corpuscle, divided into varicose branches which formed a meshwork and ended in knobs. Several other authors also observed these endings in the external genitalia. Piersol (1905) recorded that these endings were present in external genitalia. Ham (1953) described two types of bulbs in the external genitalia. The simpler one was an encapsulated granular mass in which the nerve fiber terminated at the superior pole in a small knob while the more complex variety, found in the conjunctiva, was a bulb in which the afferent nerve fiber branched repeatedly and ended in several free enlarged terminations. These endings had a structure similar to that of the Pacinian corpuscles though they were smaller and simpler in construction (Maximow and Bloom, 1957). Trautmann and Fiebiger (1957) described these nerve endings to be cylindrical bodies with from two to five lamellae. The nerve fiber lost its sheath on entering the bulb, penetrated the entire length of the finely granular and concentrically striated inner bulb and terminated in a knob. Copenhaver and Johnson (1958) reported that the end bulbs of Krause were probably the simplest of the encapsulated sensory endings, spherical or oval in shape and consisted of a thin lamellated capsule of flattened connective tissue cells and fibers surrounding a central cavity called the inner bulb. The naked axons of one or more myelinated fibers which passed through the inner bulb ended in a spherical skein-like glomerulus as in

the external genitalia. These investigators also observed that the end bulbs of Krause were located in the visceral layer of the prepuce and in the clitoris of all animals.

The Genital Corpuscles. Satterthwaite (1882) reported that these round endings were found at the base of papillae, 0.1439 to 0.2001 mm in diameter and that they presented a mulberry appearance. Bohm et al. (1904) found these endings in the deeper part of the mucosa of the glans penis and the prepuce in the male and in the clitoris and the neighboring structures in the female. They were found to be round, oval, egg, or pear-shaped or even slightly lobulated; 0.06 to 0.40 mm by 0.04 to 0.10 mm and surrounded by a relatively thick fibrous capsule of three to eight lamellae between which were irregular flattened cells with round or oval nuclei. Within the capsule was a central core of a semi-fluid substance, the nature of which was not fully known. One or two nerves entered the smaller corpuscles while from eight to 10 entered the larger ones. In each case the nerves divided dichotomously to form a loose meshwork. The medullary sheath was lost after a few turns. The neuro-fibrils divided repeatedly and presented a structure resembling a tangle of fine threads. Now and then some of the larger fibers left the corpuscle and terminated in neighboring corpuscles or passed to the epithelium where they terminated between the cells. Piersol (1905) noticed the genital corpuscles which represented a group of partly fused simple spherical end bulbs, in the integument of the glans penis and clitoris. According to Sabotta (1930) these receptors which occurred in the glans penis and the clitoris consisted of an inner bulb surrounded by several distinct sheaths. The nerves entered the corpuscles at various places and divided into finer

branches to form a plexus. Ham (1953) and Copenhaver and Johnson (1958) found that the genital corpuscles occurred in the external genitalia and that they were deeply placed in the connective tissue in the penis and the clitoris. Maximow and Bloom (1957) said that the genital corpuscles, which were similar in structure to the Pacinian corpuscles, were located in deeper connective tissue of the glans penis, in the mucous membrane of urethra and were scattered in the glans clitoris. Trautmann and Fiebiger (1957) believed that these endings resembled the Golgi-Mazzoni corpuscles, but had a thicker sheath of three to eight lamellae and a dense network of neuro-fibrils consisting of from one to 10 axons. These endings were situated in the visceral preputial layer of all animals and in the visceral membrane of the glans clitoris.

The Pacinian Corpuscles. Piersol (1905) described these endings which were widely distributed in man and mammals as elliptical, semi-transparent bodies, 2 to 3 mm by 1 to 1.5 mm. They occurred along the nerves supplying the skin, especially the hand, foot and external genitalia. The corpuscle consisted of from 25 to 50 concentric connective tissue lamellae each with an outer transverse and an inner longitudinal layer of fibers lined by a single layer of endothelial cells. The lamellae which surrounded the inner bulb were thinner and more compact than those at the periphery. Consequently the clear serous fluid enclosed between individual lamellae was larger in the latter. The adjacent lamellae were occasionally crossed by trabeculae. The myelinated fiber coursed up the intra-capsular ligament along which the lamellae were united, and lost its medullary sheath where it gained entrance into the inner bulb. The free axis cylinder terminated in a knob-like structure. The small artery which accompanied the nerve

fiber divided and distributed itself in the outer layers of the corpuscle. These special end bulbs were located in the integument of the glans and other parts of the penis and in the vagina and labia minora.

Ham (1953) and several others observed that special nerve endings were present in external genitalia. These nerve endings were located, according to Maximow and Bloom (1957), in the deeper layers of the skin, under mucous membranes, in loose connective tissue in general and also along the dorsal vein of the penis. In addition they were observed in deeper connective tissue of both the glans penis and labia minora and under the albuginea in cavernous bodies in the penis and clitoris. They were white in color and ranged from 1.0 to 4.0 mm in length and 2.0 mm in width. One or more thick myelinated fibers penetrated the corpuscle and lost the medullary sheath while the sheath of Schwann became continuous with the capsule.

Trautmann and Fiebiger (1957) described these large nerve endings, 2 mm in diameter, as having an external system of double lamellae of from 20 to 60 layers which became thinner toward the center. The lamellar space was filled with serous fluid and was crossed by collagenous fibrils. The internal lamellar system which enclosed the inner bulb comprised trough-like half-tubes of two layers each. The lamellae, through which the nerve passed, were connected by a long strand of connective tissue. The nerve fiber lost its sheath before extending into the inner bulb where it terminated either undivided or bifurcated. The axis cylinder and its terminal branches gave off numerous fine twigs along their course and formed an elongated coil. Glomeruli were formed by small blood vessels. The authors believed that a rise in blood pressure resulted in an increase in the fluid content of the lamellar spaces and distension of the lamellated corpuscles

and that the latter were probably concerned with regulation of blood pressure as well as sensation of local pressure. The endings were found in the penis, clitoris and in the follicles of tactile hairs. They were also seen in the visceral preputial layer in the cat and in the visceral membrane of the clitoris in cat and sow.

A survey of pertinent portions of the literature has revealed that several authors described the special nerve endings, to a greater or lesser extent in the human and probably mammals in general. Boeke (1932) said that the end plates varied greatly in size and form, even in different muscles of the same animal and also that no two corpuscles of Meissner were quite the same. This might be true in the case of other endings too. However, no specific mention of the occurrence of these endings in the external genitalia of the bovine was made.

Staining. The staining qualities of tissues depended generally upon the preliminary treatment, especially the fixative to which they were subjected. The degree of selectivity of silver impregnation methods was markedly influenced by the initial fixation. Bodian (1937) suggested the use of formol-trichloracetic acid-alcohol as a fixative for nerve endings. Various methods of fixation of tissues and staining of the paraffin sections had been described by several authors. Bodian (1936) recommended fixation of the peripheral nerve endings by perfusion with 80 per cent alcohol in order to prevent post-mortem autolytic changes as much as possible. Rogers (1931) used 10 to 20 per cent commercial formalin or Bouin's picro-acetoformol as a fixative, for seven days or longer in his new silver methods for paraffin sections. He described four methods of staining nerve material and claimed almost equally good results with each. Gray (1953)

listed a more simple method. Bodian (1936) reported that the selective and sharp impregnation of nerve fibers with silver depended on the chemical properties of the fiber and upon the properties of the silver solution used. The amount of ionized silver present in the solution determined the character of the impregnation, by affecting the speed of the reduction and thus the size of the silver particles available for deposition. The protargol solution contained much less ionized silver than was present in silver nitrate solution. The addition of copper did not increase the amount of ionized silver in solution but probably decreased it still further since silver was deposited on the copper in the protargol. The exact mode of reaction of the copper in producing the selective staining of nerve elements has proved as difficult a problem as any involved in the silver impregnation methods. The copper in the protargol solution was deposited along with silver in the nerve fibers.

MATERIALS AND METHODS

Five males, identified as animals A through E and seven females, identified as animals F through L of grade dairy breeding were used in this study. Tissue specimens were removed immediately after death of the animal either in the necropsy room of the Department of Pathology at Kansas State University or a commercial packing plant in Salina, Kansas.

The tissues removed from the males were the glans penis, the body of the penis just posterior to the glans, and the penile layer of the prepuce. From the females the clitoris, the adjacent vestibule, and the labia were taken. The males were 10 months (A), 18 months (B), three years (C), six years (D), and eight years (E) of age. The females were

10 months (F), one year (G), three years (H), five years (I), six years (J and K), and eight years (L) of age.

The glans penis from specimen A was collected at two levels, B at four levels, C at nine levels and D and E at five levels each. The body of the penis from A was taken at 12 different levels behind the glans penis, from B, D and E at two levels, while C was not sampled. The prepuce from A was sampled at four levels, from B and D at three levels, from E at two levels, and C was not sampled.

The clitoris was obtained at two levels from F, at six levels from G and at three levels from animals H through L. The vestibule adjacent to the clitoris was taken at two levels from F, three from G, and at one level only from the rest (H through L). The labia were collected from the inferior commissure from G, from the inferior commissure and the middle part from H through L, and at four levels from F.

Representative pieces of tissue were fixed in a solution containing 5.0 ml of formol, from 1.0 to 2.0 gm of trichloracetic acid in 90 ml of 80 per cent ethyl alcohol, (Bodian, 1937), for seven days as suggested by Rogers (1931). The tissues from animals A, C, F and G were embedded in paraffin and sections cut at 10 to 12 u. One set of tissues from each animal was stained with hematoxylin and eosin. Two sets of sections from these animals were stained with the silver method of Gray (1953). One half of those stained by Gray's method were counter-stained with eosin. Two sets of tissues taken from animals B, D, E, H, I, J, K and L were fixed as mentioned above and then washed. They were subsequently dehydrated in three per cent ammonium hydroxide solution in 80 per cent ethyl alcohol for 24 hours. Dehydration was completed by immersing the tissues

in three per cent ammonium hydroxide in 90 per cent ethyl alcohol for 24 hours. They were cleared in chloroform and embedded in paraffin. One set of sections from these specimens was stained with hematoxylin and eosin. Another set was stained by protargol method given by Bodian (1936).

A total of approximately 892 cross sections were mounted on 448 slides and examined.

RESULTS AND DISCUSSION

Bodian (1936) found that fixation by perfusion with 80 per cent alcohol proved most satisfactory. By fixation with alcohol, with formol and trichoracetic acid added, peripheral nerve endings stained brilliantly (Bodian, 1937). Rogers (1931) fixed materials in 10 to 20 per cent formalin and then dehydrated in three per cent solution of ammonium hydroxide in 80 per cent and 90 per cent alcohol for 24 hours each. For this study the materials were fixed in formol-trichloracetic-alcohol. Gray's method (1953) of placing fixed material in a three per cent solution of ammonia in 90 per cent alcohol for one day was adopted for materials from specimens A, C, F and G. These were then embedded in paraffin, and sections cut at 10 to 12 u thick. For the materials from the specimens B, D, E, H, I, J, K and L the writer modified Bodian's (1936) technique and adopted Roger's (1931) method in treating the fixed tissues with 80 per cent and 90 per cent ammoniated alcohol, each for 24 hours. Thereafter, the subsequent steps in Bodian's protargol method were followed.

In most of the section impregnation methods with silver, it was extremely difficult to control completely the course of impregnation so as to obtain constant results. Gray's technique stained the nerve fibers and

also the connective tissue fibers very dark. This was, to some extent, reduced in the sections counterstained with eosin. According to this technique after toning in gold the sections were to be placed in a two per cent solution of oxalic acid for five to ten minutes. It was observed, however, that this solution turned the sections almost black by five minutes. It was found that 10 seconds in oxalic acid gave good results. Counter-staining with one per cent acridine red solution for a few seconds was recommended, but 40 to 60 seconds was found to be most satisfactory.

The Free Sensory Nerve Endings. The nerve fibers coursing through the connective tissue divided into smaller branches and terminated in knob-like structures, the free endings. In the male these sensory endings were observed in the connective tissue of the glans penis, prepuce and also in the body of the penis. In the female they were found in the connective tissue of the clitoris, vestibule and the labia. The distribution of these endings in each of these genital organs was fairly uniform though they were relatively fewer in the body of the penis and in the vestibule than in the rest of the organs studied. The fine nerve fibers, devoid of their sheath, penetrated the epithelium and passing between the epithelial cells terminated in small knobs in the protoplasm of the cells. They were evident in the stratified squamous epithelium of the glans penis, prepuce, clitoris, vestibule and labia. The transitional epithelium of the urethra also contained these endings. Copenhaver and Johnson (1958) stated that these endings did not exist beyond the stratum granulosum in the human skin. In this study they were found even in the superficial layers of the genital epithelium. The peritrichial endings of Copenhaver and Johnson (1958) were demonstrated in the hair follicles of the labia (Plate I, Figs. 1 and 2).

The Meissner's Corpuscles. Cauna (1956) described these as the tactile corpuscles found in the hairless portions of the skin. They were probably most concerned with discriminative touch and were well developed in the human. Slight modifications of these receptors occurred according to their location and the occupation of the human. In the papillae of the genital epithelium of the bovine the typical horizontal arrangement of the tactile cells was not observed, though the axon took a spiral course. Irregularly elongated corpuscles were found in the papillae of the epithelium of the glans penis (Plate II, Figs. 1 and 2) and the prepuce in the male and in the clitoris, vestibule, and the labia of the female. The age of the animal did not seem to influence the density of these endings in any of the organs of the male or the female nor was any regional variation noted. The nerve fibers branched in the connective tissue and entered the corpuscle in the papilla and as Cauna (1956) observed the neuro-fibrils did not cross over to the next papillary segment.

The End Bulbs of Krause. These bulbs were round or oval encapsulated bodies, lamellated in structure and were located in the superficial layers of the sub-epithelial connective tissue of the glans penis and the prepuce and also in the tunica adventitia of the body of the penis in the male bovine. In the female bovine they were found in the connective tissue beneath the stratified squamous epithelium of the clitoris (Plate III, Figs. 1 and 2), vestibule and the labia. Bohm et al. (1904) recorded the size of these nerve endings as 20 to 100 u by 15 to 30 u. In the bovine they measured 20 to 100 u by 10 to 75 u in the male, and 28 to 74 u by 21 to 52 u in the clitoris, 50 to 60 u by 23 to 42 u in the vestibule and 39 to 98 u by 32 to 42 u in the labia of the female. It appeared that neither

the sex nor the age of the animal had affected their size in the genitalia. Of the capsulated endings, these bulbs outnumbered the others, by far, in both sexes. Their distribution in any of the genital organs was fairly uniform. However, there were a larger number of this type of nerve endings in the glans penis and the clitoris than in the body of the penis and the vestibule. The prepuce and the labia contained an intermediate number of them.

The Genital Corpuscles. In the bovine these tactile encapsulated endings, round, oval or even slightly lobulated in shape, were found scattered along with the end bulbs of Krause in the deeper strata of connective tissue of the external genitalia of both sexes. They were conspicuous by their size and the complex network of neuro-fibrils. The dimensions were 21 to 210 u by 16 to 175 u in the glans penis, 30 to 200 u by 24 to 122 u in the prepuce and 42 to 175 u by 35 to 70 u in the body of the penis. They measured 46 to 345 u by 49 to 225 u in the clitoris, 46 to 525 u by 45 to 300 u in the vestibule and 49 to 330 u by 26 to 52 u in the labia. The size of these corpuscles in either sex, irrespective of age, did not seem to vary much. The number of genital corpuscles was considerably less than the bulbs of Krause but was slightly more than the Pacinian corpuscles. They were not found to be concentrated in any region in any organ, though the glans penis and the clitoris were relatively rich in these endings. A considerable number of them occurred in the prepuce and vestibule. There were very few in the labia and body of the penis.

The Pacinian Corpuscles. These lamellated corpuscles, the largest in size among the sensory nerve endings, were round or oval in cross-section and elliptical and elongated in longitudinal section. They were located

in the bovine in the deeper layers of connective tissue of the external genitalia of both sexes and under the albuginea and the cavernous bodies of the penis and clitoris (Plate III, Figs. 1 and 2). Several authors recorded the size of these endings in the human from 250 by 100 u to 1.0 to 4.0 mm by 2.0 mm. In the external genitalia of the bovine they varied from 58 to 435 u by 42 to 285 u. In some of the sections the intra-capsular ligament and the intra-corpuscular blood vessels were evident. Of the sensory nerve endings associated with the genitalia, the Pacinian corpuscles were the fewest. The genital organs presented no regional variation in their corpuscular content in either the male or the female. The glans penis and the clitoris had many of these endings. The prepuce and the vestibule had very few of these endings while the body of the penis and the labia contained a greater number of them.

CONCLUSION

From the observations made in this study it was clear that sensory nerve endings were present in the external genitalia of the bovine. Boeke (1932) said that the end plates varied greatly in size and form even in different muscles of the same animal and that no two corpuscles of Meissner were quite the same. Probably this is true of the other types too. With such wide limits in variation, then, the endings in the bovine resembled those of the human, in their shape, size and structure.

The free nerve endings were located in the epithelium and connective tissue of the genitalia. Copenhaver and Johnson (1958) observed that free nerve endings did not exist beyond the stratum granulosum in the human, probably due to the thick stratum corneum. In the bovine these endings

were found even in the superficial layers of the epithelium of the glans penis. The peritrichial endings were seen in the labia of the female. While the nerve fibers took a spiral course in the Meissner's corpuscle, the characteristic arrangement of horizontal layers of tactile cells was not seen in the bovine.

The sensory nerve endings were associated with blood vessels. Receptors of the same or different types often occurred in groups. The age or sex of the animal did not appear to cause differences in the number of endings. In each of the genital organs studied, the distribution of any of these endings appeared to be fairly uniform. It was observed that the end bulbs of Krause outnumbered the genital and Pacinian corpuscles, the latter types being more or less equal. The glans penis and clitoris had a rich supply of nerve endings. The prepuce and labia had a considerable number of these, but the body of the penis and the vestibule had relatively less. Though the silver methods did not give constant results, the staining technique used was satisfactory.

SUMMARY

A study of the various types of nerve endings found in the external genitalia of the bovine was carried out on specimens obtained from several male and female animals of different ages. All the receptors described in the human and mammals in general were found in the bovine. Observations on the morphology, location and distribution of these endings in the genital organs were discussed. The staining technique employed combined the methods of Rogers and Bodian and proved satisfactory.

ACKNOWLEDGMENT

The author expresses his gratitude to his Major Professor Dr. Dean S. Folse for his advice and guidance in his work, and to Dr. M. J. Twiehaus, Head of the Department of Pathology for the facilities afforded. Thanks are due to Mrs. Sue C. Baron, Department of Pathology for her prompt co-operation in the technique in all its stages. The author is indebted to Dr. D. M. Trotter, Dr. H. T. Cier and Dr. E. H. Coles for their encouragement and suggestions.

REFERENCES

Adrian, E. D. The basis of sensation. London: Christophers, 1928.

_____. The mechanism of sense organs. Physiol. Rev. 10:336. 1930.

Bodian, D. A new method for staining nerve fibers and nerve endings in mounted paraffin sections. Anat. Rec. 65:89. 1936.

_____. The staining of paraffin sections of nervous tissues with activated protargol. The role of fixatives. Anat. Rec. 69:153. 1937.

Boeke, J. Cytology and cellular pathology of the nervous system. Section VI. Nerve endings, motor and sensory. New York: Paul B. Hoeber, 1932.

Bohm, A. A., M. von Davidoff, and G. C. Huber. A text book of histology. New York: W. B. Saunders and Co., 1904.

Cauna, N. Nerve supply and nerve endings in Meissner's corpuscles. Amer. J. Anat. 99:315-350. 1956.

Copenhaver, Wilfred, M., and Dorothy D. Johnson. Bailey's text book of histology. 14th ed. Baltimore: Williams and Wilkins Company, 1958.

Daysach, L. J. The comparative morphology of the erectile tissue of the penis with especial emphasis on the probable mechanism of erection. Amer. J. Anat. 64:111. 1938.

Gray, P. The microtomist's formulary and guide. New York: Blakiston and Co., 1953.

Ham, A. W. Histology. 2nd ed. Philadelphia: J. B. Lippincott Co., 1953.

Lambert, A. E. Introduction and guide to the study of histology. Philadelphia: Blakiston Co., 1944.

Lee, A. B. The microtomist's vade mecum. 11th ed. London: J. and A. Churchill Ltd., 1950.

Mariappa, D. The anatomy of the foetal Indian elephant. Part IX. The histology of certain organs. Ind. Vet. J. 35(9):436. 1958.

Maximow, A. A., and W. Bloom. A text book of histology. 7th ed. Philadelphia: W. B. Saunders Company, 1957.

McLeod, W. M. Bovine anatomy. Minneapolis: Burgess Publishing Company, 1958.

Piersol, G. A. Normal histology. 6th ed. Philadelphia: J. B. Lippincott Co., 1905.

Rogers, W. M. New silver methods for paraffin sections. Anat. Rec. 49:81-87. 1931.

Sabotta, J. Text book of human histology and microscopic anatomy. 4th ed. New York: J. E. Stechart Co., 1930.

Sanders, F. K. Special senses, cutaneous sensation. Annual Review of Physiology. 9:553. 1947.

Satterthwaite, T. E. A manual of histology. 2nd ed. New York: William Wood and Co., 1882.

Sisson, S. The anatomy of the domestic animals. 4th ed. Philadelphia: W. B. Saunders Co., 1953.

Trautmann, A., and J. Fiebiger. Fundamentals of histology of domestic animals. Translated by Habel, R. E., and E. L. Biberstein. 9th ed. New York: Comstock Publishing Associates, 1957.

Trotter, D. M. Histological observations of the genitalia of the immature, the castrated and the mature bovine male. Amer. J. Vet. Res. 20:213-222. 1959.

APPENDIX

EXPLANATION OF PLATE I

Sections of inferior commissure of labia, X280.

Arrows point to peritrichial endings.

Figure 1. Protargol Stain.

Figure 2. Hematoxylin and Eosin Stain.

PLATE I

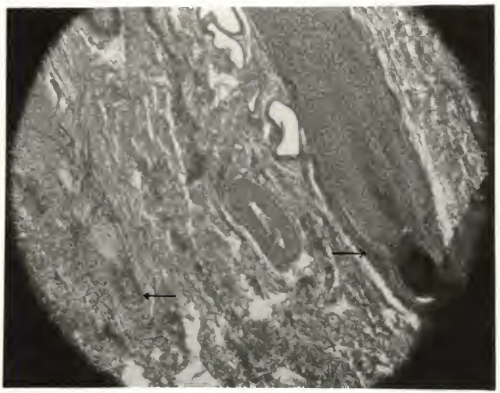

Figure 1

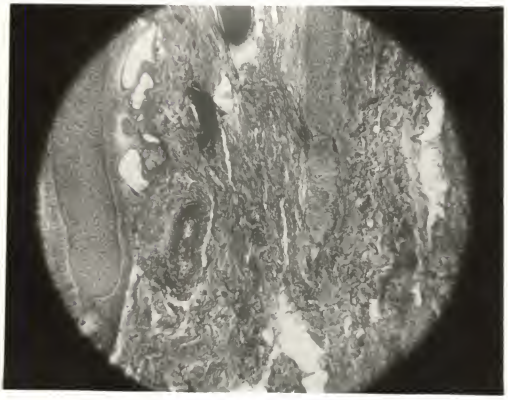

Figure 2

EXPLANATION OF PLATE II

Sections of glans penis, X280.

epi. - surface epithelium.

M.C. - Meissner's corpuscle.

t.c. - tactile cells

Figure 1. Protargol Stain.

Figure 2. Hematoxylin and Eosin Stain

PLATE II

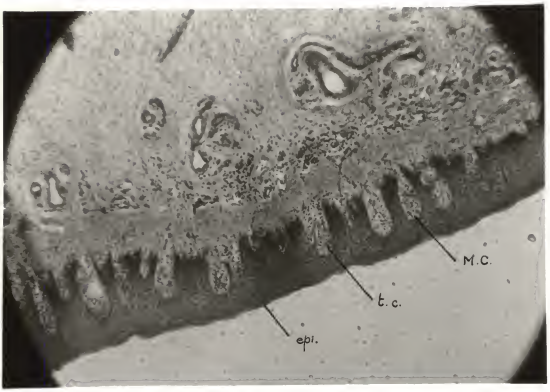

Figure 1

Figure 2

EXPLANATION OF PLATE III

Sections of clitoris, X280.

e.b.K. - end bulb of Krause.

i.c. - intracapsular ligament.

P.c. - Pacinian corpuscle.

Figure 1. Protargol Stain

Figure 2. Hematoxylin and Eosin Stain

PLATE III

Figure 1

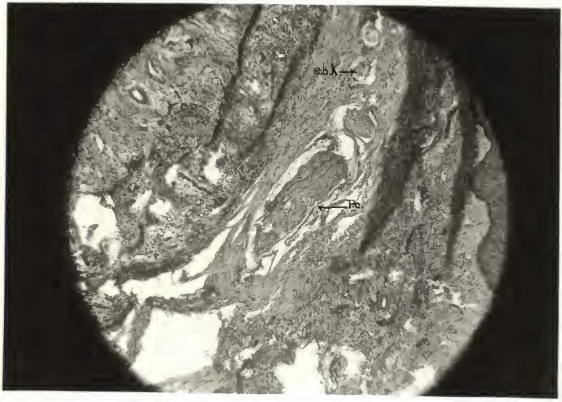

Figure 2

STUDIES ON THE NERVE ENDINGS OF THE EXTERNAL GENITALIA OF THE BOVINE

by

V. P. RAO

B. V. Sc. University of Madras, 1943.

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Pathology

KANSAS STATE UNIVERSITY
OF AGRICULTURE AND APPLIED SCIENCE

1959

Several investigators have described the sensory nerve endings in the external genitalia in the human. The only literature available is a generalization of the occurrence of these endings in mammals including the ruminant. The present work was undertaken to study the location and distribution of the various types of nerve endings in the external genitalia of the bovine. This included the glans penis, anterior part of the body of the penis, and the prepuce in the male and the clitoris, adjacent part of the vestibule and the labia in the female.

The sensory fibers of the spinal nerves terminated in the free or non-encapsulated afferent endings in the epithelium of the glans, prepuce and the urethra and also the sub-epithelial connective tissue. The sensory fibers also ended in other types of receptors such as Meissner's corpuscles located in the sub-epithelial papillae of the genitalia, the end bulbs of Krause which were found in the superficial strata of the connective tissue and the genital and Pacinian corpuscles which occupied the deeper layers of the connective tissue.

The materials were obtained at different levels, fixed in formol-trichloracetic-alcohol for seven days. Paraffin sections were cut at 10 to 12 u. Representative sections from each animal were stained by hematoxylin and eosin. Sections from some of the animals were stained by Gray's silver method while the rest were stained by Bodian's protargol method and counter-stained with acridine red. About 892 sections mounted on 448 slides were examined.

The fixative and the staining technique employed were quite satisfactory. The free nerve endings were found in the connective tissue and epithelium of the genitalia. They extended to the superficial layers of the

epithelium. The labia of the female were also supplied with peritrichial endings. It has been observed by other workers that the typical transverse striations and the horizontal arrangement of nerve fibers in Meissner's corpuscles were reduced or lost after a period of inactivity of hands and that such corpuscles resembled those on the back of the fingers, in the human. In the bovine, however, the typical horizontal arrangement of the tactile cells was not evident; the Meissner's corpuscles were found to be irregularly elongated in the sub-epithelial papillae. In addition the end bulbs of Krause were found in the superficial layers of connective tissue of the genitalia and outnumbered the other types of sensory endings. The genital and Pacinian corpuscles occupied the deeper layers of connective tissue and were found to be more or less equal in number.

Examination of the sections revealed that the glans penis and clitoris were rich in the sensory nerve endings. The prepuce and labia had fewer of these endings while the body of the penis and vestibule possessed the least number of the receptors. In each of the genital organs, however, no regional variation in density of these endings was found. The development of these endings was not as complex in the bovine as in the human. In the human, variation in Meissner's corpuscles depended on the age of the individual, type of exercise to which the corpuscles were exposed and also the constancy and regularity of their use. In the bovine there was lack of discriminative usage of these endings, however, the structure and distribution of them was found to be as in the human. This suggested the probable similarity in responses to stimuli.

PLATE III

Figure 1

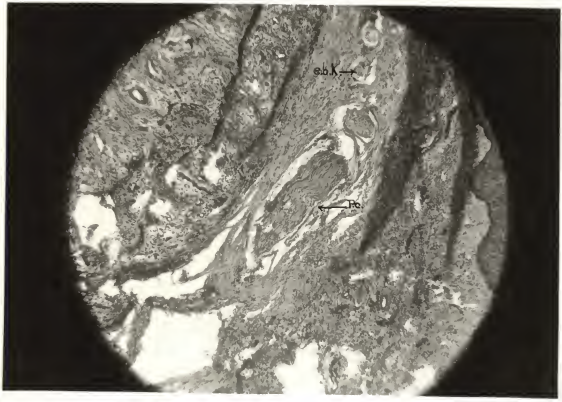

Figure 2

STUDIES ON THE NERVE ENDINGS OF THE EXTERNAL GENITALIA OF THE BOVINE

by

V. P. RAO

B. V. Sc. University of Madras, 1943.

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Pathology

KANSAS STATE UNIVERSITY
OF AGRICULTURE AND APPLIED SCIENCE

1959

Several investigators have described the sensory nerve endings in the external genitalia in the human. The only literature available is a generalization of the occurrence of these endings in mammals including the ruminant. The present work was undertaken to study the location and distribution of the various types of nerve endings in the external genitalia of the bovine. This included the glans penis, anterior part of the body of the penis, and the prepuce in the male and the clitoris, adjacent part of the vestibule and the labia in the female.

The sensory fibers of the spinal nerves terminated in the free or non-encapsulated afferent endings in the epithelium of the glans, prepuce and the urethra and also the sub-epithelial connective tissue. The sensory fibers also ended in other types of receptors such as Meissner's corpuscles located in the sub-epithelial papillae of the genitalia, the end bulbs of Krause which were found in the superficial strata of the connective tissue and the genital and Pacinian corpuscles which occupied the deeper layers of the connective tissue.

The materials were obtained at different levels, fixed in formol-trichloracetic-alcohol for seven days. Paraffin sections were cut at 10 to 12 u. Representative sections from each animal were stained by hematoxylin and eosin. Sections from some of the animals were stained by Gray's silver method while the rest were stained by Bodian's protargol method and counter-stained with acridine red. About 892 sections mounted on 448 slides were examined.

The fixative and the staining technique employed were quite satisfactory. The free nerve endings were found in the connective tissue and epithelium of the genitalia. They extended to the superficial layers of the

epithelium. The labia of the female were also supplied with peritrichial endings. It has been observed by other workers that the typical transverse striations and the horizontal arrangement of nerve fibers in Meissner's corpuscles were reduced or lost after a period of inactivity of hands and that such corpuscles resembled those on the back of the fingers, in the human. In the bovine, however, the typical horizontal arrangement of the tactile cells was not evident; the Meissner's corpuscles were found to be irregularly elongated in the sub-epithelial papillae. In addition the end bulbs of Krause were found in the superficial layers of connective tissue of the genitalia and outnumbered the other types of sensory endings. The genital and Pacinian corpuscles occupied the deeper layers of connective tissue and were found to be more or less equal in number.

Examination of the sections revealed that the glans penis and clitoris were rich in the sensory nerve endings. The prepuce and labia had fewer of these endings while the body of the penis and vestibule possessed the least number of the receptors. In each of the genital organs, however, no regional variation in density of these endings was found. The development of these endings was not as complex in the bovine as in the human. In the human, variation in Meissner's corpuscles depended on the age of the individual, type of exercise to which the corpuscles were exposed and also the constancy and regularity of their use. In the bovine there was lack of discriminative usage of these endings, however, the structure and distribution of them was found to be as in the human. This suggested the probable similarity in responses to stimuli.